Role of MLL-Wild Type in hematopoesis and leukemia transformation in MLL-Partial Tandem Duplication mouse models

Daniel L. Brook[1], Nicholas A. Zorko[1], Adrienne M. Dorrance[1], Daniel A. Yanes[1], Kelsie Bernot[1], Susan P. Whitman[1], Elshafa Ahmed[1], Kathleen McConnell[1],Patricia A. Ernst[2], Gang Huang[3], Guido Marcucci[1], and Michael A. Caligiuri[1]

1. Comprehensive Cancer Center, The Ohio State University, Columbus, OH 2. Norris Cotton Cancer Center, Dartmouth College, Hanover, NH 3. Dept. of Experimental Hematology, Cincinnati Children's Hospital, Cincinnati, OH

The James

THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER

Background

Acute Myeloid Leukemia (AML) is a blood cancer arising mostly in older adults. *MLL*-partial tandem duplication *(MLL*-PTD*)* is present in 5-7% of cytogenetically normal AML patients. *FLT3*-internal tandem duplication *(FLT3*-ITD*)* is found in 30-35% of AML patients. A subset of AML patients with both *FLT3*-ITD *and MLL*-PTD have a poor prognosis.

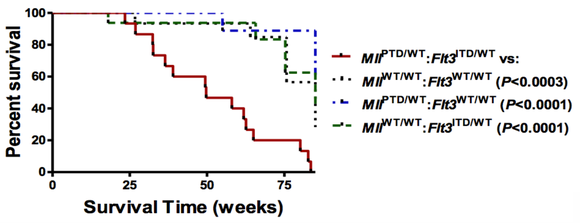
Single mutant mice expressing either *Mll-PTD* or *Flt3-ITD* independently do not develop leukemia; however, a double mutant mouse expressing *Mll-PTD and Flt3-ITD* develops AML with a long latency and mimics human AML phenotypically and molecularly[1].

MLL-WT expression is silenced in both human samples[2] and mouse models[3] of *MLL*-PTD+ AML. Germ-line absence of *Mll*-WT in MII-PTD+ ($MII^{PTD/-}$) mice is embryonic lethal[4], so an *Mll*-PTD conditional knockout mouse was obtained to generate $Mll^{PTD/FLOX}$;$Flt3^{ITD/WT}$ mice.

Objective 1: Does *Mll*-PTD maintain gain-of-function in the absence of *Mll*-WT?

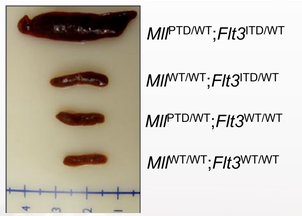
Objective 2: Is *Mll*-WT a tumor suppressor in an *Mll*-PTD+ AML murine model?

Mll-PTD;*Flt3*-ITD cooperate to induce fatal acute leukemia



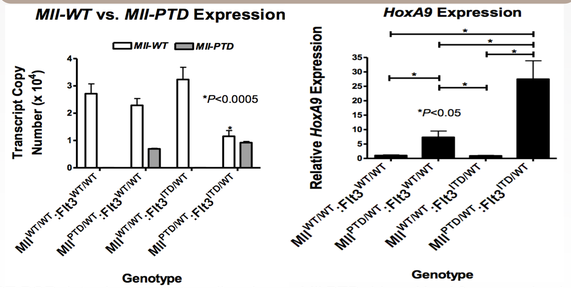
Survival curve of single knock-in and WT control mice in comparison to the heterozygous double mutant mice.

$Mll^{PTD/WT}$;$Flt3^{ITD/WT}$ mice with AML develop splenomegaly



Spleens of mice at time of harvest (80 wks).

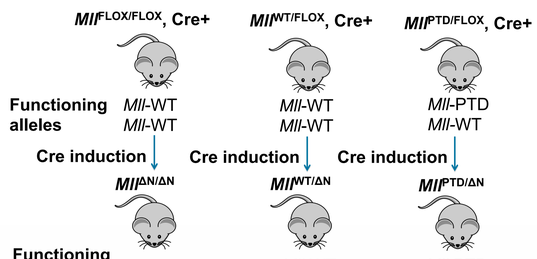
$Mll^{PTD/WT}$:$Flt3^{ITD/WT}$ murine acute leukemia is similar to human AML



RT-PCR data demonstrates silencing of *Mll*-WT expression in leukemic $Mll^{PTD/WT}$:$Flt3^{ITD/WT}$ mice.

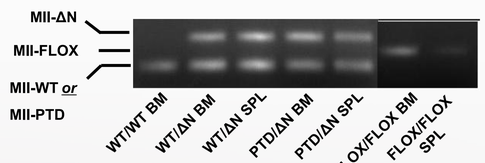
Mll-PTD drives HoxA9 expression in single and double mutant murine model.

Objective 1: Does *Mll*-PTD function without *Mll*-WT?



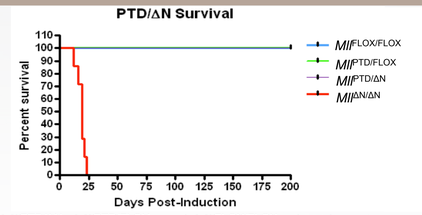
Experimental design of conditional knockout of *Mll*-WT floxed allele (*Mll*-FLOX).

Cre induction deletes *Mll*-FLOX to *Mll*-ΔN

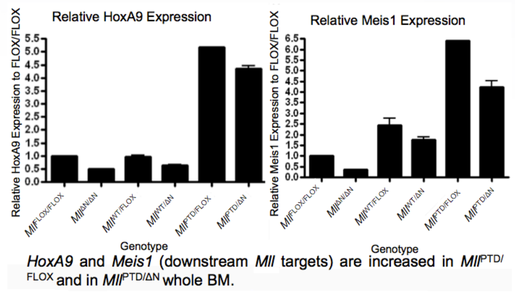


The floxed (*Mll*-FLOX) allele is completely deleted, producing *Mll*-ΔN in hematopoietic tissues after induction of Cre recombinase.

Mll-PTD acts as a gain-of-function mutation in the absence of *Mll*-WT

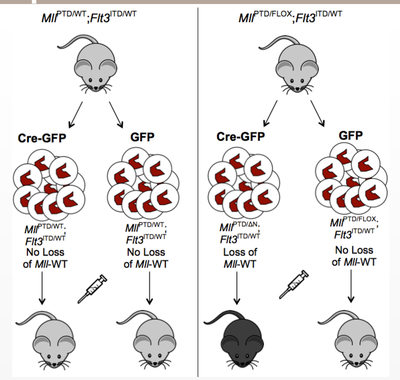


$Mll^{PTD/\Delta N}$, $Mll^{PTD/FLOX}$, and $Mll^{FLOX/FLOX}$ mice do not succumb to BM failure after deletion of *Mll*-floxed allele.



HoxA9 and *Meis1* (downstream *Mll* targets) are increased in $Mll^{PTD/FLOX}$ and in $Mll^{PTD/\Delta N}$ whole BM.

Objective 2: Is *Mll*-WT a tumor supressor in *Mll*-PTD+ AML?



Experimental design of to test whether *Mll*-WT affects leukemia transformation in *Mll*-PTD+ murine model.

Summary

1. *Mll*-PTD;FIt3-ITD mice develop a transplantable AML that is phenotypically and molecularly similar to human AML.
2. $Mll^{PTD/\Delta N}$ mice maintain normal hematopoiesis survive, but $Mll^{\Delta N/\Delta N}$ mice succumb to bone marrow failure soon after induction.
3. *Mll*-PTD appears to act as a gain-of-function mutation based on expression levels of the downstream MII targets *HoxA9* and *Meis1.*
4. Breeding of $Mll^{PTD/FLOX}$;$Flt3^{ITD/WT}$ with and without Cre mice is in progress to help evaluate the effect of *Mll*-WT on AML transformation.

Future Directions to analyze *Mll*-WT in acute leukemia model

1. Evaluate whether loss of *Mll*-WT (hypothesized to function as a tumor suppressor in *Mll*-PTD+ AML) decreases latency of leukemogenesis in an *Mll*-PTD;*Flt3*-ITD mouse model of AML .

2. Evaluate expression of oncogenic HoxA9 and Meis1 in $Mll^{PTD/FLOX}$;$Flt3^{ITD/ITD}$ mouse model pre- and post- conditional knockout.

3. Monitor disease progression by flow cytometry based immunophenotyping.

References

1. Zorko NA, et al. Mll partial tandem duplication and Flt3 internal tandem duplication in a double knock-in mouse recapitulates features of counterpart human acute myeloid leukemias. *Blood*. 2012;120(5):1130-1136.

2. Dorrance, A. M., et al. (2006). Mll partial tandem duplication induces aberrant Hox expression in vivo via specific epigenetic alterations. Journal of Clinical Investigation 116, 2707-2716.

3. Whitman, S. P., et al. (2005). The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. Blood 106, 345- 352.

4. Jude CD, et al. Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. Cell Stem Cell 1(3);doi:10.1016/ j.stem. 2007.05.019, 2007

Acknowledgements

The following OSUCCC Shared Resources were used to conduct these studies: Nucleic Acid, Flow Cytometry, Comparative Pathology and Mouse Phenotyping. This work was supported by National Cancer Institute grants to MAC (CA089341; CA009338), GM (CA102031), to MAC and GM (CA140158) to NAZ (T32 GM12453, OSU College of Medicine Fellowship and Pelotonia Graduate Fellowship), to KMB (T32 CA009338), and to DB, DY and RS (Pelotonia Undergraduate Fellowship).